Crystallographic Studies of Cell Signaling and DNA Replication	X25

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Structure of the C-terminal region of p21WAF1/CIP1 complexed with human PCNA:

The arrest of DNA replication in response to the detection of DNA damage is a fundamental mechanism by which the cell minimizes the potential transmission of lethal mutations. One mediator of this response is the protein p21WAF1/CIP1 (hereafter referred to as p21), an inhibitor of the cyclin dependent protein kinases (cdks) that control the initiation of the S phase of the cell cycle and concomitant DNA replication. An elevation of nuclear levels of the tumor suppresser protein p53 occurs upon detection of DNA damage. This stimulates transcription of p21 as part of a p53 signaling pathway in which the cessation of DNA replication is coupled to stalling of cellular mitosis.

p21 is a member of a family of cdk inhibitory proteins, including p27Kip1 , p57Kip2 and p27XIC1 that share sequence similarity in their N- terminal regions. These highly conserved regions are by themselves sufficient to inhibit cyclin-cdks that function at major transition points in the cell cycle. p21 is also able to directly inhibit DNA replication by binding tightly to and blocking the action of the DNA polymerase processivity factor PCNA (proliferating cell nuclear antigen). PCNA is a DNA tracking protein that is required for the rapid and processive replication of DNA by the polymerases d and e because it forms a sliding clamp that tethers the polymerase to DNA during strand synthesis. The C-terminal region of p21WAF1/CIP1 is distinct from that in p27Kip1 and p57Kip2, and contains a PCNA-binding sequence. The overexpression in mammalian cells of C-terminal fragments containing this region is sufficient to arrest DNA replication. The PCNA-binding functionality of p21 appears to reside entirely in the C-terminal 22 residues of the protein, which also suffice to block PCNA-mediated DNA replication directly. A peptide corresponding to these 22 residues binds to human PCNA with high affinity, comparable to that of the intact p21 protein (Kd 2.5 nM, as measured by biosensor analysis), and efficiently inhibits DNA synthesis in vitro.

Using data measured at beamline X25, we have determined the crystal structure of human PCNA complexed with a peptide corresponding to the 22 C-terminal residues of p21, determined at a resolution of 2.6 Angstroms.. p21 binds to PCNA in a 1:1 stoichiometry, with an extensive array of interactions that include the formation of a b sheet with the inter-domain connector loop of PCNA. An intact trimeric ring is maintained in the structure of the p21- PCNA complex, with a central hole available for DNA interaction. The ability of p21 to inhibit the action of PCNA is therefore likely to be due its masking of elements on PCNA that are required for the binding of other components of the polymerase assembly.

The p21-PCNA interface has the hallmarks of a tight and specific molecular interaction, and by its ability to block DNA replication it maps regions of the PCNA surface that are likely to be important for polymerase attachment. These regions on PCNA are also potential targets for the design of small molecule analogs that might mimic the action of the p21 peptide.